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13. ABSTRACT ***(Maximum 200 Words)***

A number of circulating markers have been identified that have the potential to be used in the detection or prognosis of breast cancer. Unfortunately, no single marker is consistently increased in breast cancer patients when compared with the general population. We hypothesize, however, that a sophisticated analysis of large number of circulating markers would accurately detect breast cancer as well as provide a valuable tool for prognosis. Therefore, we propose to develop a rapid and simple system to measure a large number of blood markers associated with breast cancer. We will accomplish this by developing an antibody microarray with antibodies specific to different blood markers. Currently, we have screened several markers, including PSA and HGF. We have refined the microarray to measure markers with a sensitivity down to 0.5 pg/ml. This sensitivity will allow the use of this microarray to screen up to 200 serum samples from breast cancer patients and control patients. These data will be analyzed using sophisticated computer programs that are designed to find relationships in a complex data set such as this. These studies will result in a prototype chip that can be used for the rapid determination of circulating markers associated with breast cancer.

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Introduction

Circulating blood carries chemical information from every cell in the body in the form of proteins, hormones and other factors that can potentially be assayed to screen for cancers and other diseases. In the case of breast cancer, a number of circulating markers have been identified that have the potential to be used in the detection or prognosis of the disease. Unfortunately, no single marker is consistently increased in breast cancer patients when compared with the general population. We hypothesize, however, that a sophisticated analysis of large number of circulating markers would accurately detect breast cancer as well as provide a valuable tool for prognosis. Therefore, we propose to develop a rapid and simple system to measure a large number of blood markers associated with breast cancer. We will accomplish this by developing an antibody microarray with antibodies specific to different blood markers and use this microarray to screen up to 200 serum samples from breast cancer patients and control patients. These data will then be analyzed using sophisticated computer programs that are designed to find relationships in a complex data set such as this. Once completed, these studies will result in a prototype chip that can be used for the rapid determination of circulating markers associated with breast cancer. This basic technology is likely to lead to the development of more advanced chips with wide application in screening, diagnosis, and prognosis of patients with breast cancer.

Body

In this year we have made progress toward accomplishing Task #1 (reprinted below from our approved Statement of Work).

Task 1. Design and test a diagnostic protein chip containing a repertoire (up to 25) of monoclonal antibodies specific to serum tumor markers associated with breast cancer (months 1-24).

- Develop a microarray chip containing up to 25 different antibodies that recognize circulating markers associated with breast cancer.
- Collect a preliminary number of serum sample from individuals that are apparently cancer-free and those with breast cancer. We estimate that we will have about 30-50

samples of each type by this time. These samples will be screened by Western blot methods to identify samples which have high and low levels of each targeted marker.

- Test the microarray chip using the sera identified in the above step. This will allow us to determine appropriate conditions for detection. Factors that potentially may be varied are amounts of antibodies used, either for binding to the spot or for detection; dilution of serum; incubation time; and source of antibody (some antibodies may not work satisfactorily).
- Day to day reproducibility and stability of the chips will also be determined.

Task #2 is to be done in the months 25-36 and therefore will not be addressed in this report.

The main thrust of our work this year has been directed at acquiring the antibody and antigen reagents specific for each circulating marker associated with breast cancer and developing a standard curve and sensitivity range for each marker. These values need to be determined for each marker as these ranges vary depending upon the affinity of the antibody–antigen interaction. Once the sensitivity, linear range and assay conditions are determined for each antibody-antigen combination a full antibody array can be generated.

When we began work on this project we had developed arraying methods with a Beckman Biomek robotic microarrayer. This instrument was replaced in October, 2000 with another robotic microarrayer, a Cartesian, PixSys 5500. The Cartesian robotic arrayer spots one nanoliter of a protein solution onto a 125 micron spot instead of a 200 nanoliter spot of protein that the Beckman robotic arrayer delivered. While this new spotter allows for more antibodies to be spotted on the same size microarray, it was necessary to adapt some of our methodologies to accommondate the decreased size and volume of the arrayed spot.

We have focused on three markers initially, CEA, HGF and PSA. The first two markers were initially assayed using a capture antibody-antigen-antibody sandwich format with an additional fluorescent-labeled secondary antibody used for detection. However, we have modified this assay format to a capture antibody-antigen-biotinylated antibody sandwich. The biotinylated antibody is then detected with a fluorescently-labeled streptavidin. This modification markedly improved the signal to noise ratio. Additionally, a tyramide amplification step (TSA-PerkinElmer Life Sciences, Boston, MA) was added to improve the sensitivity of the assay. In this amplification scheme the streptavidin is conjugated with HRP. HRP is used to catalyze the deposition and binding of a biotinylated tyramide onto

nearby proteins. This complex is then incubated with fluorescently labeled streptavidin and imaged. Together these assay refinements have resulted in an approximately1000-fold increase in the sensitivity of the assay (see below).

Prostrate Specific Antigen, PSA, is expressed at low levels in healthy woman (2pg/ml). However, higher levels, especially of the free form (not bound to (alpha)1-antichymotrypsin) are observed in women diagnosed with breast cancer, as high as 16ng/ml (*1*). Using antibodies to the unbound form of PSA we were able to detect PSA at a concentration of 0.3 ng/ml (Figure 1).

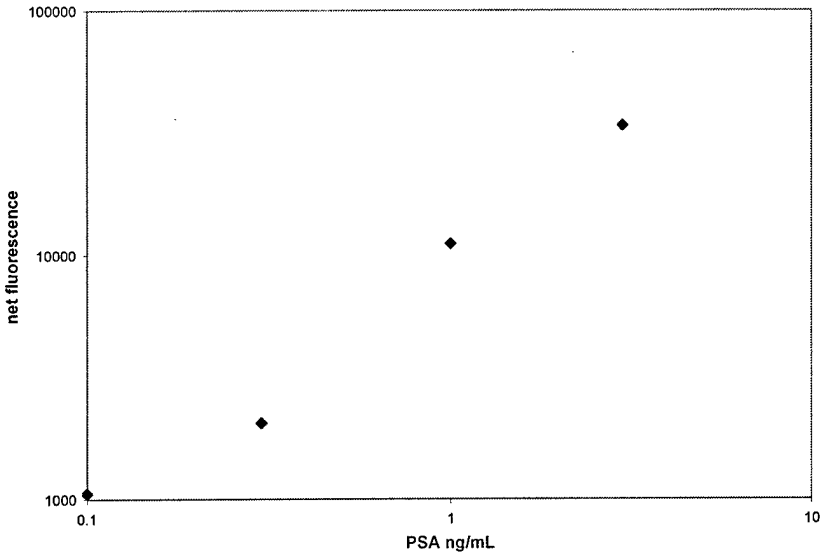


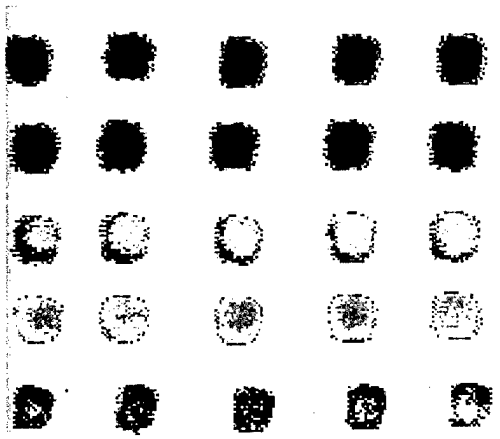
Figure 1. Detection of PSA. Antibody arrays incubated with PSA at the following concentrations, 0.1, 0.3, 1, and 3 ng/ml, were imaged using a Scan Array 3000. Log values of fluorescence detected is plotted against the concentration of PSA, also in log scale. Each data point represents the median value from five replicate samples.

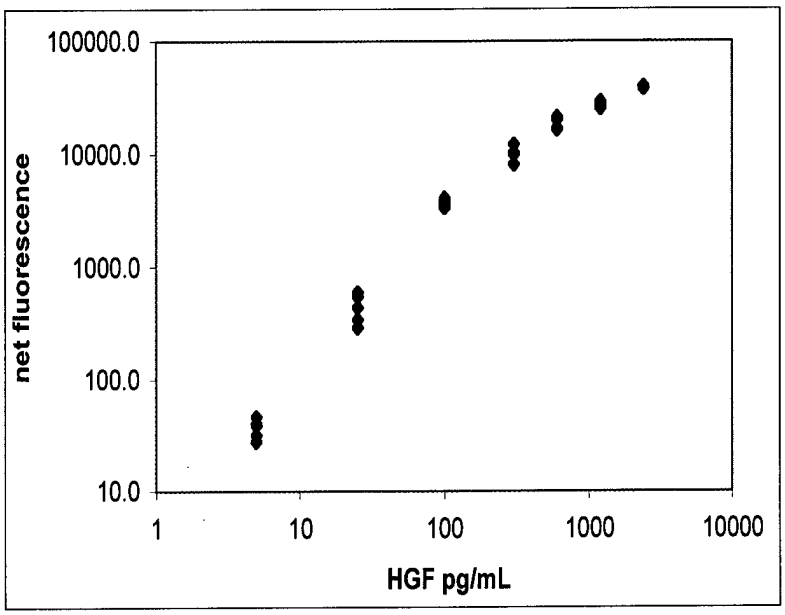
While this sensitivity is not adequate to detect PSA in normal serum it is sufficient to detect unbound PSA in serum from patients with breast cancer. It should also be noted that these experiments were performed without using the tyramide amplification. We are at present repeating these experiments using the tyramide amplification. We expect to see sensitivities in the range of 1 pg/ml concentration of PSA, which should allow us to detect PSA in normal patient's serum.

Our initial experiments with anti-CEA antibodies didn't bind with CEA antigen on a microarray. The antibodies also didn't detect CEA antigen on a Western blot, indicating that the reagents and not the microarray format are the problem. CEA is a heavily glycoslated

protein and has made analysis of the reagents difficult. We are continuing to investigate the reagents and will order new reagents if required.

Heptacyte Growth Factor, HGF, has been shown to promote cell motility and proliferation in various types of cells, including tumor cells. Additionally circulating HGF levels are frequently increased in advanced cancer patients (*2*). HGF levels in normal serum is less than 0.4 ng/ml and in woman with breast cancer it is greater than 0.4 ng/ml (*3*). Our initial assays to measure HGF did not utilize the tyramide amplification step and the sensitivity of the assay was 0.3 ng/ml.





A B.

Figure 2A. *Detection of HGF. Antibody arrays incubated with five replicate spots of HGF(from left to right) at the following concentrations from top to bottom, 10, 3, 1, 0.3, and 0.1 ng/ml were imaged with a Scan Array 3000.* B. *In another experiment antibody arrays were incubated with HGF at the following concentrations 5, 25, 100, 300, 600, 1200, and 2400 pg/ml, were imaged using a Scan Array 3000 and then plotted. Log values of fluorescence detected is plotted against the concentration of HGF, also in log scale. Each data point represents the median value from five replicate samples.*

When the assay was repeated with a tyramide amplification we were able to detect HGF at a concentration of 0.5 pg/ml, or nearly 1000-fold greater sensitivity than without tyramide. Figure 2 shows that this assay has a linear range extending from 5 pg/ml up to 1.2 ng/ml (R^2=0.97).This sensitivity and range will allow measurement of HGF concentrations in both normal and cancer patient's sera. We have analyzed the HGF concentration in one normal female's serum and determined the concentration to be between 30-100 pg/ml.

We have identified antibody and antigen sources for a number of additional breast cancer markers. There are however a handful of markers that at present are not available commercially. We intend to identify in the coming months individual laboratories that will make these antibodies or antigens available for this project.

Key Research Accomplishments

- The set-up and implementation of a new microarray robotic spotter
- Refined mAb array assay with an improved sensitivity as low as 0.5 pg/ml for HGF.
- Detection of HGF in normal female serum using a protein microarray.
- Computer training begun on the bioinformatics software, OmniVis (previously referred to as SPIRE).
- Microarray spotting is reproducible.

Reportable Outcomes

None at this time.

Conclusions

We have established a strong foundation for developing antibody microarray assays. Work this year has focused on determining the appropiate assay conditions for several antibody-antigen combinations. Having established these conditions for several markers, we will be able to quickly analyze the conditions necessary for the remaining antibody-antigen combinations. Once these are established we will be able to assemble a complete repertoire of antibodies on an array and begin to assay for the levels of cancer markers in serum samples. This type of antibody microarray has great potential for the rapid determination of circulating markers associated with breast cancer. This basic technology is likely to lead to

the development of more advanced chips with wide application in screening, diagnosis, and prognosis of patients with breast cancer.

References

1. Black MH, Giai M, Ponzone R, Sismondi P, Yu H, Diamandis, EP. 2000. Serum total and free prostate-specific antigen for breast cancer diagnosis in woman. Clin Cancer Res 6:467-73.
2. Toi M, Taniguchi T, Ueno T, Zsano M, Funata N, Sekiguchi K, Iwanari H, Tominaga T. 1998. Significance of circulating hepatocyte growth factor level as a prognostic indicator in primary breast cancer. Clin Cancer Res 4:659-64
3. Taniguchi T. Toi M, Inada K, Imazawa T. Yamamoto Y, Tominaga T. 1995. Serum concentrations of hepatocyte growth factor in breast cancer patients. Clin Cancer Res 1:1031-4.

Appendices

Personel that have worked on this project over the last year are Drs. Richard C. Zangar, Susan M. Varnum and Ron Woodbury. Curriculum vitae for each are these personel are included here.

FF Principal Investigator/Program Director(*Last, first, middle*):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Richard C. Zangar	Senior Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Western Washington University	B.S.	1982	Environmental Scie
Washington State University	M.S.	1986	Biology
Oregon State University	Ph.D.	1992	Toxicology
Wayne State University	Res. Assoc.	1992-1997	Molecular Toxicolo
Battelle, PNNL	Res. Assoc.	1997-1999	Molecular Toxicolo

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

1982-1986 Technician, Biology and Chemistry Department, Battelle, Pacific Northwest National Laboratory, Richland, Washington.

1986-1988 Technical Specialist, Biology and Chemistry Department, Battelle, Pacific Northwest National Laboratory, Richland, Washington.

1992-1997 Research Associate, Institute of Chemical Toxicology, Wayne State University, Detroit, Michigan.

1997-1999 Research Associate, Molecular Biosciences, Battelle, Pacific Northwest National Laboratory, Richland, Washington.

1999 Research Scientist, Molecular Biosciences, Battelle, Pacific Northwest National Laboratory, Richland, Washington.

2000-2001 Senior Research Scientist, Molecular Biosciences, Battelle, Pacific Northwest National Laboratory, Richland, Washington.

Awards and Honors:

1982 Student Trainee Award, Department of Energy (NORCUS)

1988-1991 Doctoral Fellowship, Department of Energy (NORCUS)

1994-1996 Research Fellowship Award, NIEHS #F32 ES05657, Title: Regulation of CYP2E1 Expression by Toxicants.

2001 Vice-President, Pacific Northwest Society of Toxicology.

Profession Activities

Co-chair of platform session "Proteomics" at the *40th Annual Meeting of the Society of Toxicology*, March 27th, 2001, San Francisco.

Chair of symposium session "Metabolizing Enzymes as Determinants of Susceptibility" at the *38th Hanford Symposium of Health and the Environment*, October 19th, 2000, Richland, WA.

Ongoing Research Projects

1999-2003 NIH/NIDDK R01 DK54812. PI: RC Zangar. *Regulation of Xenobiotic-Metabolizing CYP3A.*
This grant investigates the cell-signaling enzymes and phospholipids that regulate the stability of cytochrome P450 3A, a key enzyme in human drug metabolism. Many of the two-dimensional gel proteomics techniques proposed for the current proposal have been developed on this project. $142K directs/yr.

2000-2003 DOD/Army Breast Cancer "Idea" Award #BC990713. PI: RC Zangar. *Protein Microarray Technology for the Noninvasive Diagnosis and Prognosis of Breast Cancer*. This goal of this project is to develop a quantitative protein microarray chip for use in the analysis of protein biomarkers in serum. $75K directs/yr.

1999-2002 Internally funded Laboratory Directed Research and Development. PI: DL Springer. *Characterization of Nodes in Cell-Signaling Pathways using Proteomic Approaches*. The goals of this study involve characterization of membrane and shed proteins using Mass-Spectrometry-based proteomics. Dr. Zangar's role on this project has been to develop and optimize procedures for handling membrane proteins that are compatible with subsequent MS analyses. $375K total/yr.

2001-2004 Internal Research and Development. PI: JG Pounds. *Identification of Cancer Biomarkers using Proteomic Characterization of Serum.* The goal of this project is to develop high-throughput procedures (i.e., no gel analysis steps) for characterizing the human serum proteome to be used in identifying biomarkers of disease. Dr. Zangar's role on this project is that of an advisor/consultant, providing general guidance on sample handling and proteomic approaches. ~$3000K total/yr.

Selected Publications (1995 to present; of 32 total):

Zangar, R.C., Hernandez, M., Kocarek, T.A. and Novak, R.F. 1995. Determination of the poly(A) tail lengths of a single mRNA species in total hepatic RNA. *BioTechniques* **18,** 465-469.

Zangar, R.C., Woodcroft, K.J., Kocarek, T.A. and Novak, R.F. 1995. Xenobiotic enhanced expression of cytochrome P450 2E1 and 2B1/2B2 in primary cultured hepatocytes. *Drug Metab. Disp.* **23,** 681-687.

Zangar, R.C., Reiners, J.R. and Novak, R.F. 1995. Gender-specific and developmental differences in protein kinase C isozyme expression in rat liver. *Carcinogenesis* **16,** 2593-2597.

Zangar, R.C., Buhler, D.R. and Springer, D.L. 1995. Neonatal exposure to xenobiotics alters adult protein kinase C alpha levels and testosterone metabolism. Differential effects by neonatal diethylstilbestrol and phenobarbital. *J. Toxicol. Environ. Health.* **45,** 47-58.

Woodcroft, K.W., **Zangar**, R.C. and Novak, R.F. 1996. Methods to Evaluate Renal Cytochrome P450 as a Bioactivation System for Nephrotoxicants. In, *Methods in Renal Toxicology* (Eds. R.K. Zalups and L.H. Lash). CRC Press, Boca Raton, FL.

Runge-Morris, M., Feng, Y., **Zangar**, R.C. and Novak, R.F. 1996. Effects of hydrazine, phenelzine and hydralazine treatment on rat hepatic and renal drug-metabolizing enzyme expression. *Drug Metab. Disp.* **24,** 734-737.

Zangar, R.C., Woodcroft, K.J. and Novak, R.F. 1996. Differential effects of ciprofibrate on renal and hepatic cytochrome P450 2E1 expression. *Toxicol. Appl. Pharmacol.* **141,** 110-116.

Zangar, R.C. and Novak, R.F. 1997. Effects of fatty acids and ketone bodies on cytochromes P450 2B, 4A and 2E1 expression in primary cultured rat hepatocytes. *Arch. Biochem. Biophys.* **337,** 217-224.

Zangar, R.C., Hernandez, M. and Novak, R.F. 1997. Posttranscriptional regulation of cytochrome P450 3A. *Biochem. Biophys. Res. Comm.* **231,** 203-205.

Zangar, R.C. and Novak, R.F. 1998. Post-translational elevation of cytochrome P450 3A levels and activity by dimethyl sulfoxide. *Arch. Biochem. Biophys.* **353,** 1-9.

Cummings, B.S., **Zangar,** R.C., Novak, R.F. and Lash, L.H. 1999. Cellular distribution of cytochromes P-450 in rat kidney. *Drug Metab. Disp.* 27, 542-548.

Zangar, R.C., Okita, J., Kim, H, Edwards, R.J., Anderson, A., Springer, D.L. and Okita, R. 1999. Increased expression of cytochromes P450 3A and 2B in response to dihydropyridine calcium channel blockers. *J. Pharmacol. Exp. Ther.* 290, 1436-1441.

Thrall, K.D., Vucelick, M.E., Gies, R.A., **Zangar**, R.C., Weitz, K.K., Poet, T.S., Springer, D.L., Grant, D.M. and Benson, J.M. 2000. Comparative metabolism of carbon tetrachloride in rats, mice, and hamster using gas uptake and PBPK modeling. *J. Toxicol. Environ. Hlth.* 60, 101-118.

Cummings, B.S., **Zangar**, R.C., Novak, R.F., and Lash, L.H. 2000. Cytotoxicity of trichloroethylene and *S*-(1,2-dichlovinyl)-L-cysteine in primary cultures of rat renal proximal tubular and distal tubular cells. *Toxicol.* 150, 83-98.

Kocarek, T.A., **Zangar**, R.C. and Novak, R.F. 2000. Post-transcriptional regulation of rat CYP2E1 expression: Role of CYP2E1 mRNA untranslated regions in control of translational efficiency and message stability. *Arch. Biochem. Biophys.* 376, 180-190.

Zangar, R.C., Benson, J., Burnett, V.L., and Springer, D.L. 2000. CYP2E1 is the primary catalyst of carbon tetrachloride metabolism in human liver microsomes. *Chem.-Biol. Interact.* 125, 233-243.

Kim, H., Putt, D.A., **Zangar**, R.C., Wolf, C.R., Guengerich, F.P., Hollenberg, P.F., and Novak, R.F. 2001. Differential induction of rat hepatic cytochromes P450 3A1, 3A2, 2B1, 2B2, and 2E1 in response to pyridine treatment. *Drug Metab. Disp.* 29, 353-360.

Zangar, R.C., Kimzey, A.L., Okita, J.R., Wunschel, D.S., Edwards, R.J., Kim, H.S. and Okita, R.T. Formation of high molecular weight cytochrome P450 3A conjugates in a process distinct from the classical ubiquitination pathway. Under revision *Mol. Pharmacol.*

Zangar, R.C., Wahl, K.L., Petersen, C.E., Anderson, G.A., Rodland, K.D. and Springer, D.L. Protein primary structure determined by in-gel trypsin digestion and MALDI-MS peptide analysis. Submitted *Electrophoresis.*

Susan M. Varnum

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Richland, WA

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EDUCATION

BS Bacteriology	1980	Iowa State University, Ames, IA
Ph.D. Biology	1991	Brandeis University, Waltham, MA

RESEARCH EXPERIENCE

1980-1983 Department of Biophysics, Harvard Medical School. Isolated tumor angiogenesis factors.

1983-1985 Department of Biochemistry and Molecular Biology, Harvard University. Identification of the DNA sequences required for Gal1-Gal10 transcriptional activation.

1985-1990 Graduate student, Department of Biochemistry, Brandeis University. The mechanism for translational control of ribosomal protein mRNAs upon oocyte maturation in *Xenopus laevis*.

1991-1995 Post-doctoral fellow, Institute of Molecular Biology, University of Oregon. Molecular and genetic analysis of floral development in *Arabidopsis thaliana.*

1995-1999 Post-doctoral fellow, Molecular Biosciences Department, Battelle Pacific Northwest National Laboratory. Molecular and genetic analysis of microorganisms relevant to bioremediation. Development of methods for the immobilization of proteins onto surfaces with the goal of producing protein arrays.

1999-present Research Scientist, Molecular Biosciences Department, Battelle Pacific Northwest National Laboratory. Development of protein microarrays.

AWARDS

1986-1988	NIH Genetics Training Fellowship
1992-1996	American Cancer Society Post-Doctoral Fellowship

SELECTED PUBLICATIONS

Giniger, E., Varnum, SM, and Ptashne, M. (1985). Specific DNA Binding of Gal4, a Positive Regulatory Protein. Cell 40: 767-774.

Kreig, PA, Varnum, SM, Wormington, WM, and Melton, DA (1989). The mRNA Encoding Elongation Factor 1a (EF-1a) is a Major Transcript at the Midblastula Transition in *Xenopus*. Developmental Biology 133:93-100.

Varnum, SM, and Wormington, WM (1990). Deadenylation of maternal mRNAs during Xenopus oocyte maturation does not require specific *cis*-sequences: a default mechanism for translation control. Genes and Development 4: 2278-2286.

Varnum, SM (1991). Deadenylation and translation inactivation of ribosomal protein mRNAs during *Xenopus laevis* oocyte maturation. Doctoral thesis.

Varnum, SM, Hurney, C, and Wormington, WM (1992). Maturation-specific deadenylation in *Xenopus* oocytes requires nuclear and cytoplasmic factors. Developmental Biology 153:283-290.

Markillie LM, Varnum SM, Hradecky P, and Wong KK. (1999). Targeted mutagenesis by duplication insertion in the radioresistant bacterium Deinococcus radiodurans: radiation sensitivities of catalase (katA) and superoxide dismutase (sodA) mutants. J Bacteriol 181:666-9

Varnum SM, Cheng RC, Monroe P. Generation of a protein microarray for the simultaneous detection of analytes. Manuscript in preparation.

Varnum SM, Petersen C, Wahl K, and Springer, D. Antibody affinity arrays for the identification of protein with MALDI_TOF MS. Manuscript in preparation.

Ronald L. Woodbury

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Education:

Ph.D. Genetics and Cell Biology, Washington State University 2000
Dissertation: Protein Interactions Along the SecB-mediated Protein Export Pathway in *Escherichia coli.*

B.S. Molecular Biology, Microbiology Emphasis, Chemistry Minor, Brigham Young University
1994
Microbiology Department Scholarships May 1993 to April 1994

Summary of Qualifications:

Diverse use of biochemical, biophysical, and molecular biology techniques. Solid experience in protein chemistry. Able to work independently and as part of a team.

Experience:

Biochemistry
-Protein purification of recombinant proteins from *E. coli*
-Preparative and analytical column chromatography including HPLC,and FPLC
-Chemical modification of proteins
-Protein cross-linking
-Characterization of steady state enzyme kinetics
-Membrane preparation (*E. coli*)
-Membrane receptor protein binding assay
-*In vitro* protein translocation assay
-Isolation of starch or phytoglycogen from maize
-Native and SDS polyacrylamide gel electrophoresis
-Immunoblotting

Biophysics
-Total intensity light-scattering
-Preparative centrifugation
-Fluorescence spectroscopy

Molecular Biology
-Plasmid construction, subcloning
-Random and site-directed mutagenesis
-Heterologous gene expression
-DNA sequencing

Employment History:
2000

Graduate Research Assistant **May 1994 to Dec**

Washington State University, School of Molecular Biosciences
NIH Biotechnology Training Fellowship
Co-expressed heterologous genes for large and small subunit of ADP-glucose pyrophosphorylase from potato tuber in *E. coli*. Randomly mutagenized the large subunit gene. Determined Michaelis-Menton constants of substrates for mutants. Characterized solution interactions and membrane receptor bindingof *E. coli* SecB and SecA and mutants of each. Overexpressed and purified proteins by ammonium sulfate fractionation, ion exchange,
hydrophobic interaction, size exclusion, and affinity chromatography (HPLC and FPLC). Evaluated protein-protein interactions in solution and at membrane receptors. Verified arrangement of subunits in SecB tetramer by native PAGE.

Industrial Intern **May 1996 to September**

1996

Pioneer Hi-bred International, Des Moines, Iowa
Starch quality and biochemistry

Publications:
C.A.

R. L. Woodbury, T.B. Topping, D.L. Diamond, D.S. Suciu,

Kumamoto, S.J.S. Hardy, and L. L. Randall, Complexes Between Protein Export Chaperone SecB and SecA: Evidence for Separate Sites on SecA Providing Binding Energy and Regulatory Interactions, submitted.

T. W. Okita, T. W. Greene, M. J. Laughlin, P. S. Salamone, R. Woodbury, S. Choi, H. Ito, H. Kavakli and K. Stephens, Engineering Plant Starches By The Generation Of Modified Plant Biosynthetic Enzymes in *Engineering Crop Plants for Industrial End Uses* (1998).

T. W. Greene, R. L. Woodbury, and T. W. Okita, Aspartic Acid 413 is Important for the Normal Allosteric Functioning of ADP-Glucose Pyrophosphorylase, *Plant Physiol.* 112:1315-1320 (1996).